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6.	McKay	Res. Vet Sci (1981) 30(3): 261-265
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Research in Veterinary Science, 1981, 30, 261-265.

Location and secretion of gastric intrinsic factor in the sheep

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The site of production and secretion of intrinsic factor (IF) in the sheep has been studied using a human autoantibody directed against IF. Immunofluorescent studies indicated the abomasal parietal cell was the source of IF in the sheep. Concentrations of IF in pure gastric secretion of sheep were relatively stable at 3 to 4 iu/ml and it was estimated that the total abomasal output of IF was 10,000 to 23,500 iu per 24 hours.

GASTRIC intrinsic factor (IF) is a glycoprotein produced in the gastrointestinal tract of mammals which binds to vitamin B_{12} and facilitates its absorption from the intestine. In most species IF is present in the glandular mucosa of the body of the stomach with the exception of the pig where it is found in the pyloric and duodenal mucosa (Jefferies 1967). The cells responsible for the secretion of IF vary according to the animal studied. The parietal cell is the source of IF in man, rhesus monkey, cat, rabbit and guinea pig. In the rat and mouse it is found in the peptic cells of the fundic glands and in the pig in pyloric and Brunner's glands (Jefferies 1967). Information on the site of production of IF in the ruminant appears to be restricted to the report of Hoedemaeker (1965) who found this to be the parietal cell in the cow.

The secretion of IF has been studied mainly in human subjects where it is continuously secreted in the absence of, and increased markedly by, gastric secretory stimuli and pharmacological agents that increase gastric acid secretion. The increase on stimulation reaches its maximum almost immediately and then rapidly declines suggesting that IF is preformed and subsequently released from mucosal stores (Jefferies 1967). The secretion of IF in ruminants has not yet been examined, although vitamin B_{12} is known to be essential and is assumed to be absorbed in the small intestine after microbial synthesis in the rumen (Underwood 1977). It therefore seemed pertinent to assess the ability of ruminants to secrete IF and in the present experiments we investigated which cells were responsible for the secretion of IF in sheep and compared the secretion of IF in the gastric juice of sheep with that of man.

Identification of cells

The indirect sandwich technique of Coons et al (1955) was used to identify specific cells by immunofluorescence.

Tissue

Tissues of the reticulum, rumen, omasum, antral and body regions of the abomasum and duodenum were obtained from local abattoirs within two hours of slaughter. They were cut into 5 to 6 mm cubes and snap frozen in liquid nitrogen vapour and stored emersed in liquid nitrogen until tested.

Antisera

A mitochondrial antiserum was obtained from two human patients with primary biliary cirrhosis. The antibody was non-organ, non-species specific reacting with cells containing mitochondria and was used as a reactive control for the immunofluorescence experiments.

A parietal cell antiserum displaying antibody titres in excess of 1:100 was obtained from two human patients with chronic atrophic gastritis without pernicious anaemia. This serum did not react with IF and was absorbed twice with sheep kidney homogenate and tested by immunofluorescence to ensure there was no non-specific reaction.

An IF antiserum was obtained by venepuncture of a 13-year-old girl suffering from pernicious anaemia. The antiserum had an antibody titre in excess of 1:200. This serum also contained a weak parietal cell antibody which was removed by absorption with hog fundus homogenate and the absence of the parietal cell antibody was checked by the lack of fluorescence of hog parietal cells (these cells do not contain IF). This serum was further absorbed with sheep kidney homogenate to eliminate non-specific reactions. The reactivity of the anti-IF serum was quenched by the addition of excess pure IF.

Fluorescent goat anti-human immunoglobulins

This reagent was obtained commercially (Behringwerke Lab) and contained a fluorescence:protein ratio of 4:1. The conjugate was used at an optimal dilution of 1:16.

Immunofluorescence staining procedure

Blocks of tissue were mounted on a cryostat chuck using an inert embedding matrix (Tissue Tek OCT compound, AMES/EBOS). Sections 6 nm thick were cut on an AMES cryostat and mounted on chemically clean glass slides. Each slide was dried for 20 minutes in a stream of dry air at room temperature. Aliquots of specific antisera were allowed to thaw and reach room temperature before testing. The appropriate serum containing the antibody was carefully applied to the tissue sections and incubated for 30 minutes at 20°C in a moist chamber. The slides were then rinsed three times with phosphate buffered saline (PBS) at pH 8·6 to remove non-specific unbound proteins. Excess PBS was carefully wiped away from the tissue sections with clean dry lint-free tissue paper. Immediately after wiping each slide was flooded with two or three drops of fluorescent conjugate and reincubated in the moist chamber for a further 30 minutes. The slides were then rinsed and washed three times in PBS, dried and finally mounted in buffered glycerol before examination for specific antigen localisation. The preparations were examined with a Reichert Immunopan microscope using a Toric widefield dark ground condenser (numerical aperture 1·18/1·42) and illuminated by a 12 volt, 100 watt quartz iodine light source.

Measurement of IF

The concentration of IF in gastric secretion of sheep and man was estimated by radioimmunoassay based on the method of Ardeman and Chanarin (1963) using albumin coated charcoal as absorbent (Gottlieb et al 1965).

Briefly, the amount of IF in a standard volume of gastric juice was assessed by its ability to bind radiolabelled vitamin B_{12}. The binding of B_{12} by IF was abolished by prior addition of anti-IF antibody, whereas binding of IF by non-IF binders was unaffected. The difference in binding of gastric juice with and without anti-IF antibody was a measure of IF content of the gastric juice.

Radiolabelled vitamin B_{12}

^{57}Co-vitamin B_{12} with a specific activity of 2 μCi/μg was obtained from the Radiochemical Centre. This reagent was diluted with non-radiolabelled vitamin B_{12} to provide a working solution of 100 ng B_{12}/ml with an activity of 0·125 μCi/μg.

Neutral AB serum

AB sera from three humans were pooled and absorbed with human and sheep red blood cells to remove non-specific antibodies. This serum was checked before use to ensure it had low B_{12} binding activity not related to IF.

Anti-IF antibody

The IF-antiserum was the same as that used for the immunofluorescence studies. A four-fold dilution of this serum neutralised 50 units of IF. One unit of IF is defined as that which will combine with 1 ng vitamin B_{12}.

IF assay

The assay was set up in triplicate with the appropriate combination of saline, gastric juice, IF-antiserum and normal AB serum. The amount of standard labelled vitamin B_{12} used was 50 ng and 5 ng for human and sheep gastric juice respectively, appropriately diluted with non-labelled vitamin B_{12} to give 12,000 to 15,000 cpm in an automatic well-type gamma scintillation counter (Parkard).

Collection of gastric juice

Pure fundic secretion was collected at 15 minute intervals for four hours and at 24 hour intervals from isolated pouches of the abomasum of sheep (McLeay and Titchen 1974). The volume was recorded and estimates of titratable acidity made by electrometric titration to pH 7 using an automatic titration apparatus (Radiometer). Aliquots of secretion were neutralised with 10 M sodium hydroxide and stored at −20°C until assayed for IF.

Three normal human subjects were fasted for 24 hours and basal secretion collected by stomach tube for 60 minutes. Following intramuscular pentagastrin (6 μg/kg) gastric juice was collected at 15 minute intervals for 90 minutes. Mucus and food particles were removed from each sample by centrifugation at 4500 rpm for 15 minutes, and the samples were treated in the same manner as sheep gastric juice.

Results

Tissues from each region of the stomach and the duodenum reacted positively with the anti-mitochondrial antibody. The reaction was strong with those cells containing many mitochondria and this is illustrated by the abomasal parietal cell (Fig 1).

The anti-parietal cell antibody reacted positively only with parietal cells of the body region of the abomasum (Fig 2). No fluorescence was obtained from the other areas of the gastric and intestinal mucosa.

Positive fluorescent staining with the anti-IF antibody occurred in the body of the abomasum only and the cells reacting to the antibody (Fig 3) were parietal cells as confirmed by the reactions of the same type of cells with the anti-mitochondrial and anti-parietal cell antibodies (compare Figs 1, 2 and 3).

Fluorescence was not obtained from any of the tissues when absorbed neutral human AB serum

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FIG 1: Immunofluorescence of abomasal body tissue with anti-mitochondrial antibody. Parietal cells react most strongly. ×320

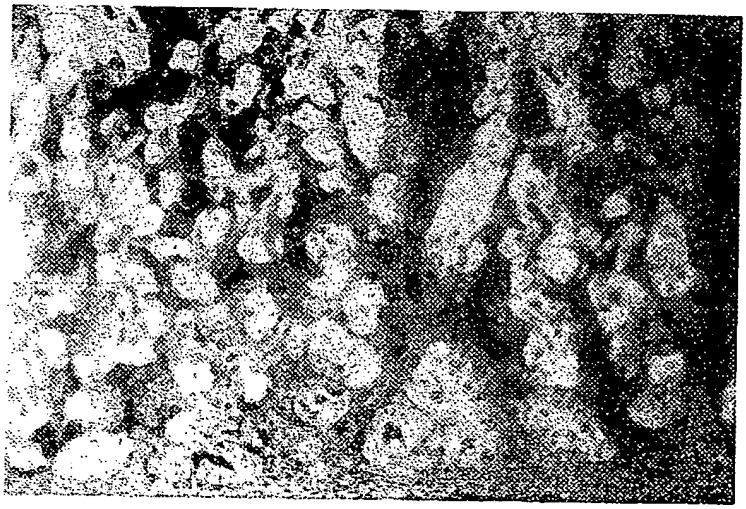

FIG 2: Immunofluorescence of abomasal body tissue with anti-parietal cell antibody. Parietal cells react strongly. ×320

replaced specific antibody containing serum in the reactions (Fig 4).

Measurement of IF

The concentration of IF of pure fundic pouch secretion of sheep was relatively stable when collected over short (15 minute) and long (24 hour) periods. Secretion collected at 15 minute intervals had an IF concentration of 3·87 ± 0·13 iu/ml (mean ± SEM, n = 8) and in 24 hour collections the concentrations were 3·62 ± 0·12 iu/ml (n = 9). In 24 hour collections the output (volume × concentration) of IF varied but this simply reflected the variation in the volume of secretion. In sheep 2 the volume of secretion collected ranged from 600 to 850 ml per 24 hours and the mean output of IF from the pouch was 2598 ± 81 iu per 24 hours (n = 4). In sheep 3 similar volumes of 850 to 1050 ml per 24 hours were collected and the mean output was 3526 ± 162 iu per 24 hours (n = 4). The surface areas of the pouches in sheep 2 and 3 were 25 and 15 per cent respectively of the surface areas of the abomasal body and it was estimated that the total abomasal secretion of IF was 10,400 and 23,500 iu per 24 hours respectively.

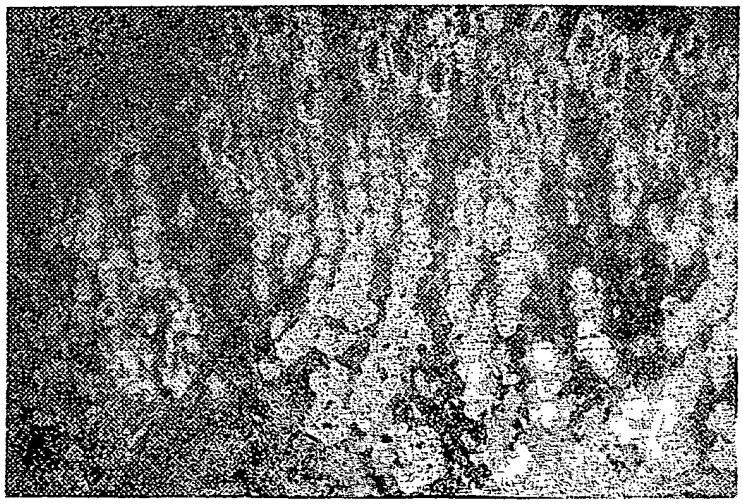

FIG 3: Immunofluorescence of abomasal body tissue with anti-intrinsic factor antibody. The parietal cells show a positive reaction with this antibody. × 150

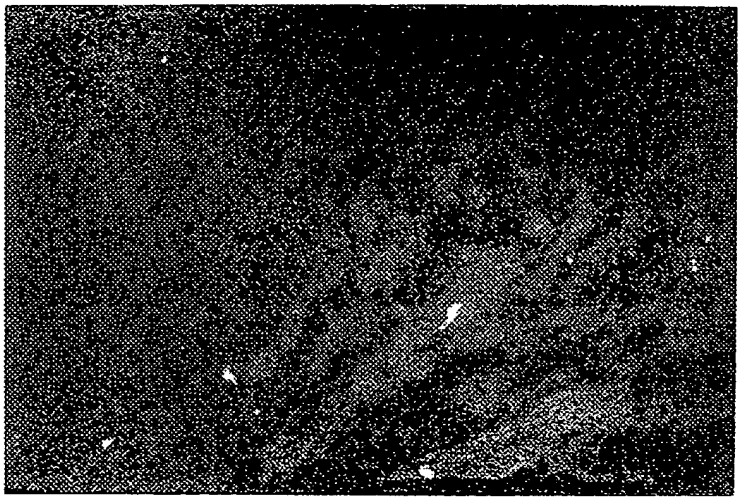

FIG 4: Negative reaction of abomasal body tissue and human AB serum without specific antibodies. × 150

The IF concentration in human gastric secretion was greater than that in sheep gastric juice. In three humans fasted for 24 hours the concentration ranged from 3·2 to 8·9 iu/ml and following stimulation with pentagastrin reached maximal concentrations of 11.5 to 38·7 iu/ml in the first 15 minutes and declined to prestimulation levels within 45 minutes of pentagastrin administration.

Discussion

The immunofluorescence studies showed that human anti-IF antibody reacted only with tissue from the body of the abomasum. The cells in this tissue reacting with anti-IF antibody corresponded to parietal cells as revealed by their reactions with the anti-mitochondrial and anti-parietal cell antibodies. Confirmation of the specificity of the IF-antibody was obtained by quenching the reaction with purified IF. These observations show that like those of the cow (Hoedemaeker 1965), the abomasal parietal cells of the sheep contain IF.

Further support for the parietal cell being the source of IF in the sheep was obtained by the presence of IF in pure gastric secretion collected from isolated pouches

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of the body of the abomasum. The concentration of IF in pouch secretion was remarkably stable at relatively low levels (3 to 4 iu/ml) compared with the maximal levels (39 iu/ml) in the pentagastrin stimulated human secretion but the amount secreted varied markedly with the volume of secretion. In our human studies the maximal levels of IF were observed within 15 minutes of stimulation and rapidly fell thereafter despite continued acid secretion. These observations support the concept that, in the human, IF is preformed and stored in the mucosa before its release (Jefferies 1967). This contrasts with the situation in the sheep. In the ruminant abomasal secretion is continuous due to the continual passage of digesta into the abomasum from the forestomach (Hill 1955, 1960). Judged on the persistently high concentrations of acid in abomasal secretion (McLeay and Titchen 1970, 1974, 1975) the parietal cells must be subjected to frequent and strong stimulation and in the sheep there would be little change for mucosal stores of IF to accumulate and subsequently give rise to high concentrations on their release.

Despite the low concentrations of IF present in sheep gastric juice the total amount produced was substantial. Estimates of the abomasal output, taking into account the relative surface areas of the pouch and abomasal body, ranged from 10,500 to 23,500 iu per 24 hours. This would be enough IF to combine with 11 to 24 μg vitamin B_{12} per day (one unit of IF combines with 1 ng vitamin B_{12}). Smith and Marston (1970) assessed the minimal total requirement of vitamin B_{12} for their sheep at 11μg per day which falls within our calculated supply of IF. However, the absorption of vitamin B_{12} from the intestine has been reported to be as low as 5 per cent of that produced in the rumen (Smith and Marston 1970) and substances other than vitamin B_{12} bind to IF and reduce the effectiveness of vitamin B absorption (Underwood 1977). Whether there is sufficient IF to allow satisfactory absorption of vitamin B_{12} by this mechanism is not clear from our studies and will require more detailed investigations.

Acknowledgements

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